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#10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent of

Ishizuka et al.

U.S. Patent No. 4,241,057

Issued: December 23, 1980

Assignee: Takeda Chemical Industries, Ltd.

Serial No. 19,185

Filed: March 9, 1979

For: ANTIBIOTIC COMPOSITIONS

SUBMISSION OF APPLICATION FOR EXTENSION OF PATENT TERM

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Filed herewith is an original and a certified duplicate of an application for extension of term of U.S. Patent No. 4,241,047. A check in the amount of $550.00 is enclosed.

Applicants generally authorize payment of any required fee for the filing of this paper (even if different from any calculation above) to our Deposit Account No. 23-0783.

Correspondence should be mailed to the undersigned at:

WEGNER & BRETSCHNEIDER
P. O. Box 18218
Washington, DC 20036-8218
(202) 887-0400
Attorney Docket No. 8700-4156A

050 02/27/89 4241057 2 111 550.00 CK

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Atty. Doc.: 8700-4156A
DATE: February 23, 1989
DPM:ldc/2.19

Respectfully submitted,

Douglas P. Mueller
Reg. No. 30,300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent of

Kenzo Ishizuka et al.

U.S. Patent No. 4,241,057

Issued: December 23, 1980

Assignee: Takeda Chemical Industries, Ltd.

Serial No. 19,185

Filed: March 9, 1979

For: ANTIBIOTIC COMPOSITIONS

APPLICATION FOR EXTENSION OF PATENT TERM

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

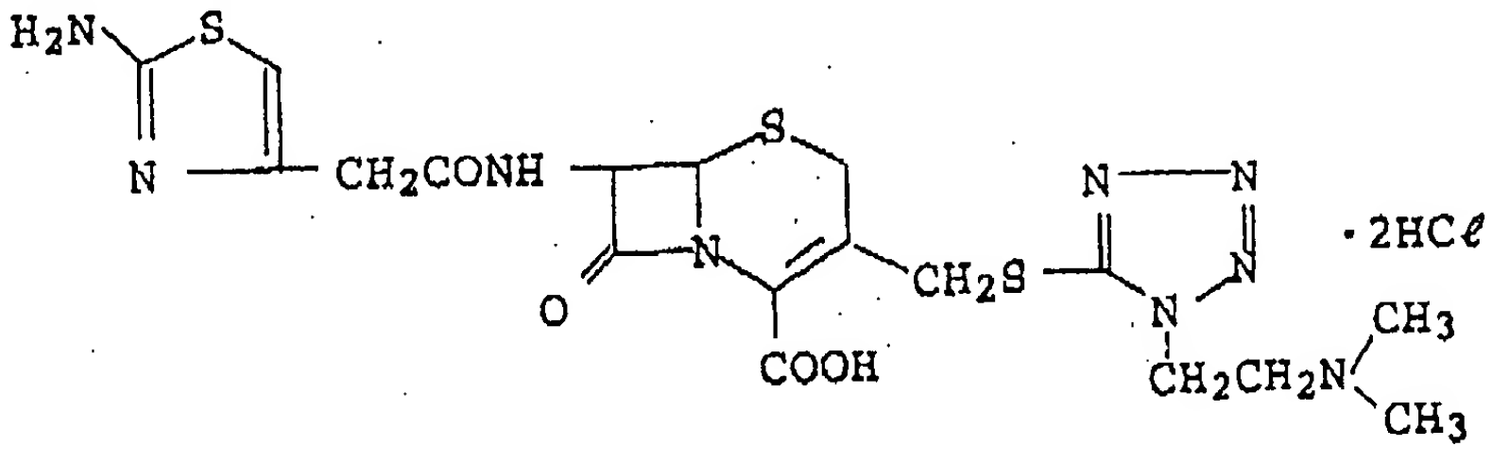
Applicant, Takeda Chemical Industries, Ltd., owner of the patent identified above, hereby applies for extension of the term of the patent.

IDENTIFICATION OF COPENDING APPLICATION

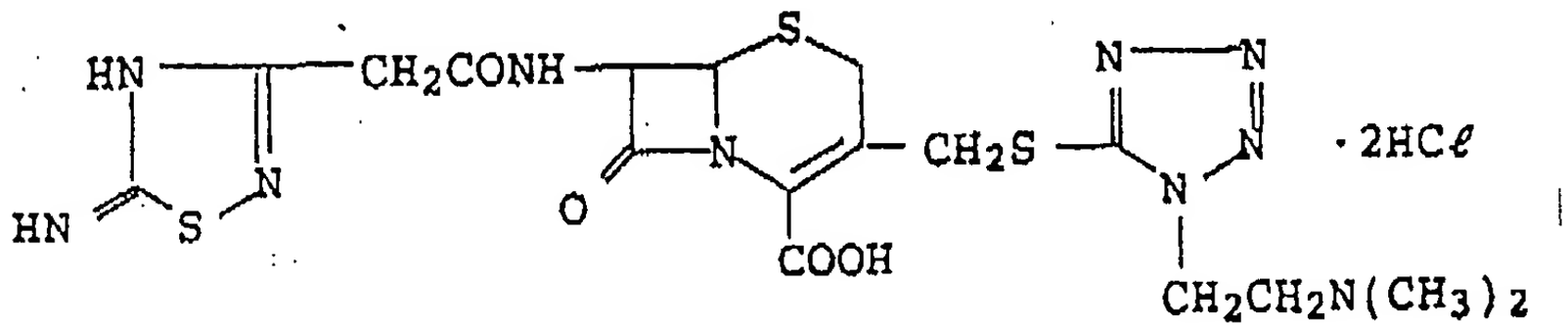
It should be noted that filed concurrently with this application is an application for extension of the term of U.S. Patent No. 4,161,527, based upon the same regulatory review period as the present application. In the event both of these applications are deemed allowable, the application for extension of U.S. Patent No. 4,161,527 will be abandoned. Neither patent has expired or been previously extended. No other patent has been extended for this regulatory review period.

THE APPROVED PRODUCT

The approved product, known as CERADON TM, is an antibiotic for intravenous administration. The active ingredient has the generic name cefotiam dihydrochloride. The chemical name is 7-beta [2-(2-aminothiazol-4-yl)acetamido]-3-[[[1-(2-dimethyl-aminoethyl)-1H-tetrazol-5-yl]thio]methyl]-ceph-3-em-4-carboxylic acid, dihydrochloride. The empirical formula is $C_{18}H_{23}N_9O_4S_3.2HCL$, with a molecular weight of 598.54. The structural formula is as follows:



The above thiazole form of the active ingredient is in a tautomeric relationship with the thiazoline form shown below.



The thiazoline form has the name 7beta-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride.

CERADON TM also includes 242 mg sodium carbonate per gram of cefotiam activity. 1.138 g cefotiam dihydrochloride is required

for 1 g of cefotiam activity. This corresponds to 1.2 equivalents of sodium carbonate per equivalent of cefotiam dihydrochloride.

CERADON TM is provided in reduced pressure vials. The pressure is less than about 50 mm Hg.

THE STATUTE UNDER WHICH THE REGULATORY REVIEW OCCURRED

Review occurred under Section 507 of the Federal Food, Drug and Cosmetic Act (21 USC 301 et seq).

DATE OF PERMISSION FOR COMMERCIAL MARKETING OR USE

FDA approval for CERADON TM was granted on December 30, 1988.

IDENTIFICATION OF EACH ACTIVE INGREDIENT

The sole active ingredient in CERADON TM is cefotiam dihydrochloride. This is the first approval for commercial marketing or use under the Federal Food, Drug and Cosmetic Act.

SUBMISSION OF THIS APPLICATION

This application is being submitted within the 60-day period permitted for submission, the expiration of the 60-day period being February 28, 1989.

THE PATENT FOR WHICH EXTENSION IS SOUGHT

The patent for which extension is sought is U.S. Patent No. 4,241,057, issued December 23, 1980, expiring December 23, 1997. The named inventors of this patent are Kenzo Ishizuka, Hiroshi Fujisawa and Etsunosuke Noda.

COPY OF THE PATENT

A copy of the patent is provided herewith as Attachment 1.

DISCLAIMER, CERTIFICATE OF CORRECTION, RECEIPT OF MAINTENANCE FEE PAYMENT OR REEXAMINATION CERTIFICATE

The patent copy mentioned above includes the Certificate of Correction issued on May 11, 1982 for this patent. There are no disclaimers, receipts of maintenance fee payment or reexamination certificates.

CLAIMS LITERALLY READING ON THE APPROVED PRODUCT

Claims 1 and 2 of U.S. Patent No. 4,241,057 read literally on the approved product.

Claim 1

Claim 1 reads as follows:

A vacuum-sealed vial, which contains a solid antibiotic composition comprising 7ß-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride or its hydrate and sodium carbonate or sodium hydrogen carbonate, the ratio of the hydrogen chloride moiety of 7ß-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride or its hydrate relative to said sodium carbonate or sodium hydrogen carbonate being substantially 1:1 to 2 equivalents, the pressure in the vial being in the range of from 0 to 300 mm Hg.

Claim 1 first requires "a vacuum-sealed vial, which contains a solid antibiotic composition". The present product includes the antibiotic cefotiam dihydrochloride and is supplied as a powder in a reduced-pressure vial.

Claim 1 next requires the composition to comprise 7ß-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride or its hydrate and sodium

carbonate or sodium hydrogen carbonate. This is the thiazoline tautomeric compound of Cefotiam dihydrochloride, the active ingredient of the approved product. As noted above, the thiazoline form of cefotiam dihydrochloride of claim 1 is in a tautomeric relationship with the thiazol form. Thus, both are present. In any event, as noted at column 5 of the patent, the present claims cover both the thiazoline form and the thiazol form, its tautomeric twin. Sodium carbonate is present in the composition.

Claim 1 next requires that the equivalent ratio of the cefotiam dihydrochloride to the sodium carbonate be in the range of 1:1 to 1:2 equivalents. As described above, the equivalent ratio is 1:1.2, well within the range required by claim 1.

Finally, claim 1 requires the pressure in the vial to be under 300 mm Hg. This limitation also is met, since the pressure is less than 50 mm Hg.

Claim 2

Claim 2 reads:

A vacuum-sealed vial as claimed in claim 1, wherein sodium carbonate is contained in the composition.

Sodium carbonate is contained in the composition, thus meeting the further requirement of this dependent claim.

RELEVANT DATES OF REGULATORY REVIEW

The IND application for this product was effective May 10, 1980. The application was assigned IND No. 17404.

The NDA was submitted on April 30, 1985 and assigned NDA No. 50-601 on May 13, 1985. The NDA was approved on December 30, 1988.

DESCRIPTION OF ACTIVITIES DURING REVIEW PERIOD

During the testing or IND period, the significant dates and activities were as follows:

Date	Activity
04/09/80	Ciba Geigy submitted original IND with protocols for clinical studies 01 and 02
04/10/80	Official IND receipt date
04/30/80	FDA letter acknowledging receipt and assigning IND #17404
04/30/80	Submitted conforming amendments from Takeda
05/10/80	IND became effective
06/23/80	Submitted protocols for clinical studies 03 and 04
10/02/80	Submitted protocol for clinical study 05
11/07/80	FDA letter commenting on stability testing, specifications and assays required
12/03/80	Submitted protocols for clinical studies 06 and 07
12/11/80	Submitted report S-2-408 (Process and Packaging of SCE 963 vials), Certification of Analysis, Microbiologic Assay Procedure (Revised)
03/18/81	Submitted clinical program outline, sample protocols and Case Report Forms (Phase II-Protocol 09; Phase III-Protocols 13 and 15), summary of foreign clinical trials, request for a meeting
04/20/81	Submitted response to FDA letter of 11/07/80 including stability, potency and safety tests
04/30/81	Meeting with FDA regarding proposed development and design of Phase II and Phase III protocols

05/22/81	Submitted Annual Progress Report; Preclinical (Microbiology) Reports, Literature, Bibliography, Labelling, Clinical (Foreign Phase I Summary Data, Phase I Studies Progress Reports)
06/10/81	Submitted Phase I Safety Data for Protocols 01 through 07
07/28/81	Submitted Protocols for clinical studies 09, 13 and 14
11/11/81	Submitted Protocol for clinical study 15
12/11/81	Submitted Protocol for clinical study 10
05/06/82	Submitted Annual Progress Report; Preclinical, Bibliography, Clinical, Stability Data
12/21/82	Notified FDA that IND sponsorship transferred to Takeda Chemical Industries, Japan, effective 12/31/82
01/11/83	Notified FDA that G.H. Besselaar Associates (GHBA) of Princeton, NJ will be contract research organization and act as U.S. agent for Takeda
08/03/83	End of Phase II meeting at FDA with G.H. Besselaar Associates and Takeda representatives
09/21/83	Submitted Annual Progress Report
09/27/83	Submitted minutes of 8/3/83 meeting
06/11/84	Submitted Protocol Amendment for clinical study 15
04/30/85	Submitted Antibiotic Form 5 (NDA)
04/30/85	Official NDA receipt date

05/13/85 FDA letter acknowledging receipt and assigning NDA #50-601

Note: Periodic amendments to IND included submission of documentation for new or additional investigators as well as notifications of serious adverse experiences including patient deaths.

During the review or NDA period, the following significant activities occurred:

Date	Activity
07/26/85	Requested conversion of DMF 4565 (Cefotiam hydrochloride for injection) to a Form 6
12/08/85	Advised by FDA that data in NDA should be reorganized and that NDA should be withdrawn and resubmitted after reorganization
12/10/85	NDA withdrawn
12/12/85	Submitted Annual Progress Report of activity under IND
12/18/85	Meeting at FDA with G.H. Besselaar Associates and Takeda to discuss reorganization of clinical data for resubmission of NDA
04/11/86	Submitted Annual Progress Report of activity under IND
04/30/86	Resubmitted NDA 50-601 following reorganization of clinical data
06/12/86	Resubmitted request to have DMF 4562 (Cefotiam hydrochloride bulk product) converted to a Form 6
06/12/86	Submitted request to have DMF 4565/6292 (Cefotiam hydrochloride for injection) converted to a Form 6
08/07/86	Meeting with FDA to discuss manufacturing issues

09/04/86	Submitted report "Development of Separate Filling Method for Cefotiam Dosage Form" dated July 1986 as an Amendment to Form 6 Application No. 62-633 (formerly DMF 4565/6292)
09/11/86	Advised by FDA to reanalyze data for Protocol 14A omitting Dr. Reynolds' data
09/22/86	DMF 4562 for manufacture of Cefotiam hydrochloride (bulk) designated as Form 6 and assigned file number 62-632
10/08/86	Submitted samples of impurities and analytical data
10/23/86	Submitted manufacturing amendment to Form 6 (Application 62-633)
10/23/86	Submitted Analytical and Sterility data requested by FDA
02/06/87	Submitted revisions to NDA requested by FDA including label and labelling, foreign safety data, reanalysis of Protocol 14A
04/22/87	Submitted Annual Progress Report of activity under IND and foreign safety data
05/12/87	Submitted, at FDA's request, justification for the format chosen for reorganization of clinical data in NDA
02/11/88	FDA requested further explanation of 5/12/87 letter
03/15/88	Submitted further justification to support letter of 5/12/87 and requested a meeting with FDA
08/19/88	Meeting with FDA to discuss Protocol 9 and status of NDA
09/26/88	Submitted Annual Progress Report of activity under IND and foreign safety data
10/03/88	Submitted data requested by FDA regarding penicillinase-producing organisms and methicillin resistant organisms
11/08/88	Submitted Safety Update Report

12/01/88	Meeting with FDA (Biopharmaceutics)
12/06/88	Submitted revised package insert and pharmacokinetic data requested by FDA
12/08/88	Submitted newly revised package insert and revised labels
12/14/88	Submitted certain reanalyzed pharmacokinetic data from Protocol 02
12/23/88	Submitted revised package insert and pharmacokinetic reports B 92/1981 and CRB R9/1981
12/29/88	Submitted letter of agreement concerning pharmacokinetic issues, batch certification and proposed monograph
12/30/88	Submitted final package insert revised according to FDA instructions
12/30/88	NDA approved

ELIGIBILITY FOR EXTENSION

In the opinion of applicant, U.S. Patent No. 4,241,057 is eligible for an extension of two years. The length of this extension is determined as follows:

IND Phase - May 10, 1980 through April 30, 1985 - 1,817 days

U.S. Patent No. 4,241,057 issued December 23, 1980, 227 days after the effective date of the IND application. Subtracting this from 1,817 and dividing by 2 yields an extension attributable to the IND application of 795 days.

NDA - April 30, 1985 to December 30, 1988 - 1,340 days

Adding the periods available for the IND application and the NDA yields a total of 2,135 days.

Since this patent issued before September 24, 1984 and the request for IND exemption was filed before September 24, 1984, no more than a two-year extension is available. The two-year extension would provide an expiration date of December 23, 1999, which is earlier than the date obtained by adding 14 years to the approval date, December 30, 2002. Therefore, an extension of two years is believed to be in order.

DUTY OF DISCLOSURE

Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought herein.

FEE

A check in the amount of $550.00 accompanies this application in payment of the fee set forth in 37 CFR 1.20(n).

POWER OF ATTORNEY

Applicant hereby appoints Harold C. Wegner, Reg. No. 25,258 and Douglas P. Mueller, Reg. No. 30,300 to prosecute this application, to transact all business in the Patent and Trademark Office in connection therewith and to receive the Certificate of Extension.

All correspondence should be sent to: Douglas P. Mueller, Wegner & Bretschneider, P. O. Box 18218, Washington, DC 20036-8218.

All telephone calls should be directed to: Douglas P. Mueller, (202) 887-0400.

DECLARATION

The undersigned declares that:

(1) He is an officer of Takeda Chemical Industries, Ltd., Applicant, and is authorized to execute this application for extension of U.S. Patent No. 4,241,057 on behalf of said Applicant, pursuant to 37 CFR 1.740(b)(1); and that said Applicant is the owner of U.S. Patent No. 4,241,057;

(2) He has reviewed and understands the contents of this application for extension of U.S. Patent No. 4,241,057;

(3) He believes U.S. Patent No. 4,241,057 is eligible for extension under 37 CFR 1.710;

(4) He believes an extension of two years is justified under 35 USC 156 and the applicable regulations;

(5) He believes U.S. Patent No. 4,241,057 meets the conditions for extension of the term of a patent as set forth in 37 CFR 1.720; and

(6) He hereby declares that all statements made herein of my his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent term extension issued thereon.

DATE: Feb. 17, 1989

Hiroshi Iwata

Hiroshi Iwata
Director, General Manager
of Patent & Licensing Department
Takeda Chemical Industries, Ltd.

ATTACHMENT 1

U.S. PATENT NO. 4,241,057

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Patent of

Kenzo Ishizuka et al.

U.S. Patent No. 4,241,057

Issued: December 23, 1980

Assignee: Takeda Chemical Industries, Ltd.

Serial No. 19,185

Filed: March 9, 1979

For: ANTIBIOTIC COMPOSITIONS

CERTIFICATION

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

It is hereby certified that the attached is a true copy of the application for extension of term of U.S. Patent No. 4,241,057, the original of which is filed herewith.

Respectfully submitted,

Douglas P. Mueller
Reg. No. 30,300

WEGNER & BRETSCHNEIDER
P. O. Box 18218
Washington, DC 20036-8218
(202) 887-0400

Attorney Docket: 8700-4156A
Date: February 23, 1989
DPM:ldc/2.19

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,241,057

DATED : December 23, 1980

INVENTOR(S) : Kenzo Ishizuka, Hiroshi Fujisawa et al.

It is certified that error appears in the above—identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page Insert:

-- (30) Foreign Application Priority Data

August 31, 1976 Japan 51-104582 --.

Signed and Sealed this

Eleventh Day of May 1982

Attest:

Ruth M. Hroy

Attesting Officer

GERALD J. MOSSINGHOFF

Commissioner of Patents and Trademarks

United States Patent [19]

Ishizuka et al.

[11] **4,241,057**

[45] **Dec. 23, 1980**

[54] **ANTIBIOTIC COMPOSITIONS**

[75] Inventors: **Kenzo Ishizuka, Amagasaki; Hiroshi Fujisawa, Toyonaka; Etsunosuke Noda, Yao, all of Japan**

[73] Assignee: **Takeda Chemical Industries, Ltd., Japan**

[21] Appl. No.: **19,185**

[22] Filed: **Mar. 9, 1979**

Related U.S. Application Data

[62] Division of Ser. No. 828,841, Aug. 29, 1977, Pat. No. 4,161,527.

[51] **Int. Cl.**[3] **A61K 31/545**

[52] **U.S. Cl.** **424/246**; 206/524.8

[58] **Field of Search** 206/524.8; 424/246

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

823861 6/1975 Belgium 424/246

Primary Examiner—Jerome D. Goldberg
Attorney, Agent, or Firm—Wenderoth, Lind & Ponack

[57] **ABSTRACT**

Solid antibiotic compositions containing 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)-ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride or its hydrate and a pharmaceutically acceptable carbonic acid salt are rapidly converted into an injectable solutions by the addition of a solvent, and the injectable solution shows high antibacterial activity with less local reactions when injected.

7 Claims, No Drawings

ANTIBIOTIC COMPOSITIONS

This is a division, of application Ser. No. 828,841, filed Aug. 29, 1977 now U.S. Pat. No. 4,161,527, issued on July 17, 1979.

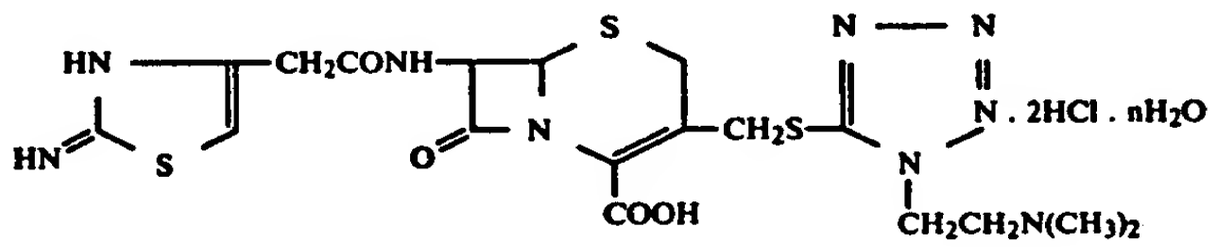
The present invention relates to solid antibiotic compositions containing 7β-[2-(2-imino-4-thiazolin-4-yl)-acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyi-3-cephem-4-carboxylic acid dihydrochloride or its hydrate as an effective ingredient and a pharmaceutically acceptable carbonic acid salt as an additive. The composition of the present invention is used for preparation of injectable solution which is of value for the treatment of diseases in animals including domestic fowls and human being, particularly for prevention or therapy of the infectious diseases caused by Gram-positive and Gram-negative bacteria in those animals or of value as an antiinfectious agent or a disinfectant, for example, for surgical instruments or hospital rooms. The compound "7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylaminc)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride" may be hereinafter abbreviated simply as "TTC".

TTC and its hydrates are new compounds which have strong antibacterial activity against Gram-positive and Gram-negative bacteria and is stable in storage. However, when TTC or its hydrate is intramuscularly injected, there are encountered the necrosis of muscle cells, discoloration or brown degeneration of the local tissue, hyperemia and other local reactions at the sites of injection. Thus, improvements have been needed in these aspects. Moreover, while TTC or its hydrate must be dissolved in a solvent such as distilled water before it may be administered through injection, it is rather slow to be dissolved, this being another disadvantage which has had to be overcome. The present invention did thorough analysis of the above problems and have now found that the antibacterial activity of TTC is not impaired in the presence of the pharmaceutically acceptable carbonic acid salt; that if a solvent such as distilled water is added to a mixture of TTC or its hydrate and a pharmaceutically acceptable carbonic acid salt, carbon dioxide gas is evolved and the dissolution of the medicament is considerably hastened by its agitating effect; and that the aforementioned local reactions are decreased where the solution thus obtained is administered through injection. The above findings were followed by further studies, on which basis this invention has been conceived and developed.

TTC or its hydrate, the starting material for the composition of this invention, can be easily produced, for example by reacting hydrogen chloride with 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid or the corresponding sodium salt, which acid and salt are described in Belgian Pat. No. 823861 and Dutch Pat. Application No. 7416609, in the presence or absence of water, recovering TTC or its hydrate from the reaction mixture after the reaction and, if desired, drying the product. The reaction may be effected in accordance with the salt formation reaction or the neutralization reaction between a base and an acid, the reaction having hitherto been well known among chemists in the field of cephalosporins. The reaction is usually carried out in a solvent or a mixture of solvents. The solvent may be the above-mentioned water, an organic solvent or a mixture thereof. The organic solvent is preferably acetone, ethanol, n-propanol, iso-propanol, methyl ethyl ketone, tetrahydrofuran, etc. The amount of hydrogen chloride to be reacted is usually 2 to 6 mols per mol of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid and 3 to 7 moles per mol of the corresponding sodium salt. The reaction is normally carried out at temperature in the range of from −10° C. to 40° C. The reaction usually goes to completion within 5 hours. After the reaction, TTC or its hydrate is recovered from the reaction mixture by per se conventional procedure such as lyophilization or concentration of the reaction mixture, precipitation of TTC or its hydrate by the addition of less soluble solvent such as the above-mentioned organic solvent, etc.

When the reaction is carried out in a reaction system which does not contain water, thus obtained product is usually TTC (anhydrous). The anhydrous product may be converted into the corresponding hydrate of TTC. On the other hand, when the reaction is carried out in a reaction system containing water, the product is collected from the reaction mixture usually in the form of hydrate of TTC. The hydrate may be made into TTC for example by means of drying.

The cephalosporins (i.e. TTC and its hydrate) are shown by the formula:



, in which n is a number in the range of $0 \leqq n \leqq 6$, including anhydrate (n=0), monohydrate (n=1), dihydrate (n=2), trihydrate (n=3), tetrahydrate (n=4), pentahydrate (n=5) and hexahydrate (n=6) as well as compounds such that less than one mol of water is attached to any of said anhydrate and hydrates. The number n is preferably a number in the range of from one to four and the most preferably in the range of from one to two in view of stability. In this regard, it is to be understood that very small amount of organic solvent may be attached to TTC or its hydrate when organic solvent is used for the preparation of TTC or its hydrate as solvent, and it is to be construed that TTC or its hydrate having such small amount of organic solvent is covered by TTC or its hydrate throughout this specification and claims.

As examples of the pharmaceutically acceptable carbonic acid salt, there may be mentioned alkali metal hydrogen carbonates such as sodium hydrogen carbonate, potassium hydrogen carbonate, etc.; alkaline earth metal hydrogen carbonates such as magnecium hydrogen carbonate; alkali metal carbonates such as sodium carbonate, potassium carbonate, etc.; and alkaline earch

metal carbonates such as magnesium carbonate, calcium carbonate, etc. The use of any of said alkali metal carbonates and alkali metal hydrogen carbonates has the advantage of a reduced pain of injection. The alkali metal hydrogen carbonates and alkaline earth metal hydrogen carbonates have the advantage that because they give rise to twice the volume of carbon dioxide gas as compared with alkali metal carbonates and alkaline earth metal carbonates when the composition of this invention is dissolved, the composition containing TTC or its hydrate is dissolved faster.

The antibiotic composition of this invention is produced by admixing TTC or its hydrate with a pharmaceutically acceptable carbonic acid salt by means which are conventional per se. In this admixing procedure, there may also be incorporated certain other known pharmaceutical additives including local anesthetics such as lidocaine hydrochloride, mepivacaine hydrochloride, etc. TTC or its hydrate, a pharmaceutically acceptable carbonic acid salt and other pharmaceutical additives are normally used in powdery or crystalline form and the composition of this invention is normally solid.

The proportion of TTC or it hydrate relative to a pharmaceutically acceptable carbonic acid salt is such that the ratio of hydrogen chloride as a moiety of TTC or its hydrate to the pharmaceutically acceptable carbonic acid salt is within the range of normally about 1:1 to 2 equivalents and preferably about 1:1 to 1.4 equivalents. It follows that the monoacidic base such as sodium hydrogen carbonate is normally used in a proportion of about 2 to 4 mols, preferably about 2 to 2.8 mols, per mol of TTC or its hydrate and that the diacidic base such as sodium carbonate is normally employed within the range of about 1 to 2 mols, preferably 1 to 1.4 mols, per mol of TTC or its hydrate.

The composition thus produced is usually aseptically packed into vials which are then vacuum-sealed and stored. By this procedure, not only is oxidative decomposition prevented but it is rendered easy to fill the vials with a solvent for the preparation of injections, at the time of use. As the solvent, e.g. distilled water, physiological saline or an aqueous solution of a local anesthetic, is infused into the vial, carbon dioxide gas is evolved to considerably hasten the dissolution of the medicament, quick dissolution being possible even under standing condition. Filling the plenum within the vial with carbon dioxide gas precludes oxidative decomposition, permitting us to store the TTC solution obtained in the form of a solution. The proportion of said solvent for dissolution is normally about 0.5 to 100 ml., preferably about 1 to 20 ml. per gram of TTC or its hydrate in terms of TTC.

Thus the present invention provides also vacuum-sealed vial in which the above-mentioned solid antibacterial composition containing TTC or its hydrate and a pharmaceutically acceptable carbonic acid salt is vacuum-seeled. It is preferable that volume of the vial satisfies the following equation:

$$V = \frac{P_1 V_0 + 6.236 \times 10^4 AT}{P_1 - P_2}$$

in which

V is a vial volume in terms of ml.;

P_1 is a pressure in the vial after filling the vial with the solvent in terms of mmHg;

P_2 is a pressure in the vial before filling the vial with the solvent in terms of mmHg;

A is molar amount of TTC or its hydrate in the vial;

V_0 is volume of a solvent to be used for preparation of an injectable solution in terms of ml.; and

T is an absolute temperature showing ambient temperature.

The pressure in the vial before filling the vial with the solvent represented by P_2 is usually the pressure of vacuum sealing, and it is normally in a range of from about 0 to 300 mmHg and preferably in the range of from about 0 to 100 mmHg.

The pressure in the vial after filling vial with the solvent represented by P_1 is usually in the range of from 600 to 1520 mmHg, preferably in the range of from 760 to 1140 mmHg.

The molar amount of TTC or its hydrate in the vial represented by A largely depends on the use of the resultant solution. For example, in case of injection for the therapy of infectious diseases caused by bacteria in man, it is usually in the range of from 1×10^{-4} to 6×10^{-3} mol.

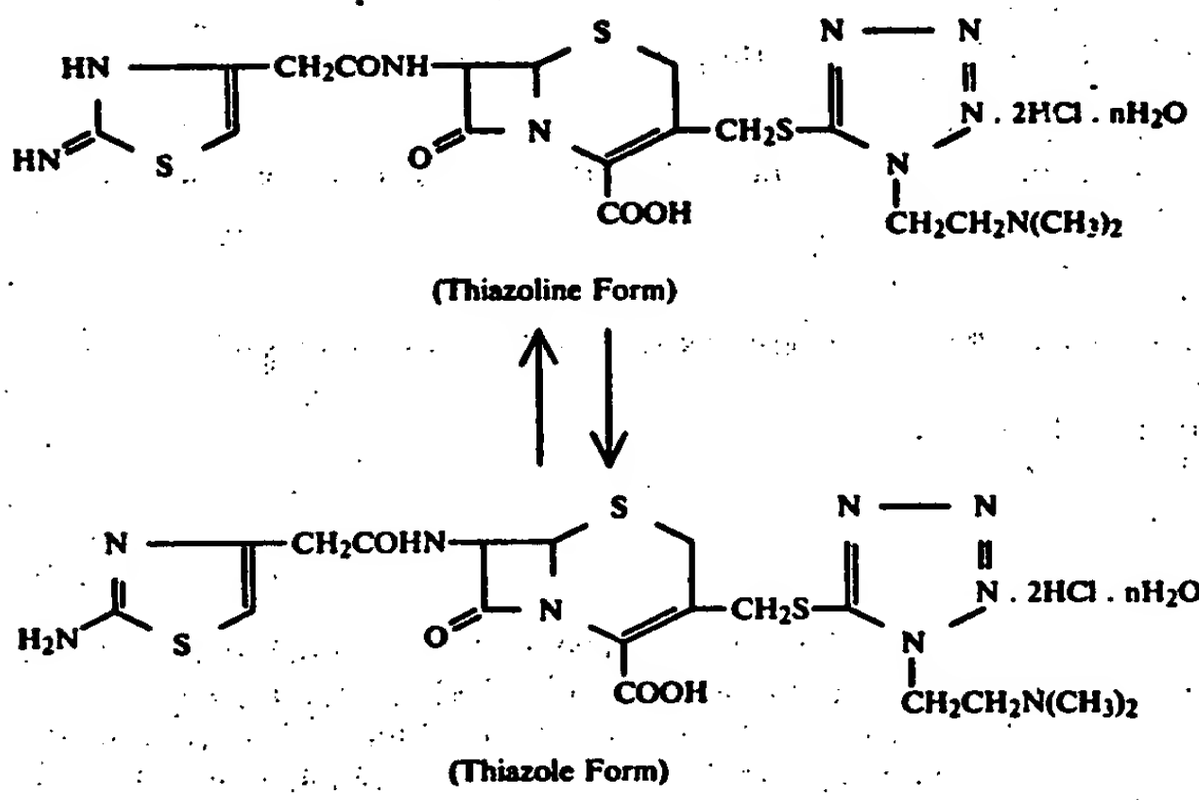
The range and preferable ranges of the volume of the solvent, i.e. the ranges of V_0, are above mentioned.

In this regard, it should be understood that the aforementioned TTC solution may be obtained by adding a solution of the pharmaceutically acceptable carbonic acid salt in the aforementioned solvent to TTC or its hydrate, optionally incorporated with any one of other conventional pharmaceutical additives.

The TTC solution thus obtained may not only be used as external disinfectants or aseptics such as disinfectants for surgical instruments, hospital rooms, drinking water, etc. but also be intramuscularly or intravenously administered as drugs for the treatment of infectious diseases in warm-blooded animals including human beings, mice, rats and dogs as caused by Gram-positive bacteria (e.g. *Staphylococcus aureus*) or Gram-negative bacteria (e.g. *Escherichia coli, Krebsiella pneumoniae, Proteus vulgaris, Proteus morganii*).

For the purpose of using the composition as an external disinfectant for the disinfection of surgical instruments, there is prepared an aqueous solution of the composition containing 100 μ/ml. of TTC, which may then be sprayed over the instruments. For the therapy of urinary tract infections in mice or human beings as caused by *Escherichia coli*, the TTC solution is intramuscularly or intravenously administered at the daily dose level of about 5 to 50 mg./kg. of TTC on an anhydrous TTC basis in three divided doses a day.

TTC or its hydrate may assume a couple of tautomeric forms by the tautomerization depicted below.

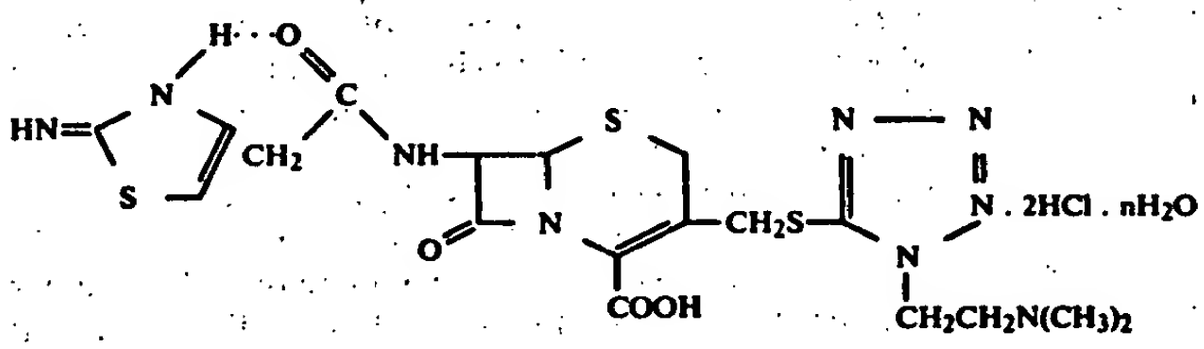


(Thiazoline Form)

(Thiazole Form)

, in which n has the same meaning as defined above.

Much inquiry has heretofore been made into the modes of existence of compounds of this type and the literature refers to the thiazoline form under certain conditions [Acta Crystallographica 27, 326 (1971] and the thiazole form under other conditions [Chemistry and Industry, 1966 ed., p. 1634]. However, various determinations have shown that TTC or its hydrate seems to predominantly assume the thiazoline form, because this form is stabilized by a contributory effect of hydrogen bonding as shown by the following formula.



in which n has the same meaning as defined above. However, this kind of equilibrium is liable to shift rather easily under the influence of various factors, e.g. the pH and polarity of the solvent used, temperature, etc., to which TTC or its hydrate may be subjected. Thus, TTC or its hydrate may be designated in accordance with whichever of the two forms. In this specification and the claims appended thereto, however, TTC and its hydrate are designated by their thiazoline forms. However, TTC and its hydrate in this invention should be construed to cover all the above tautomers.

Throughout the specification, "minimum inhibitory concentration", "gram(s)", "kilogram(s)", "liter(s)", "milligram(s)", "milliliter(s)", "percent", "Karl Fischer's method", "infrared", "nuclear magnetic resonance", "minute(s)", "calculated", "centimeter(s)", "microgram(s)", "singlet", "broad singlet", "doublet", "triplet" and "double doublet" may be abbreviated as "M.I.C.", "g.", "kg."l.", "mg.", "ml.", "%", "K.F. method", "I.R.", "N.M.R.", "min.", "calcd.", "cm.", "mcg.", "s", "bs", "d", "t" and "dd", respectively.

Reference Example 1

The antibacterial potency (M.I.C.) and toxicity of TTC

(1) Antibacterial spectrum (agar dilution)
Staphylococcus aureus FDA 209 P: 0.39 mcg./ml.
Staphylococcus aureus 1840: 0.78 mcg./ml.
Escherichia coli NIHJ JC-2: 0.2 mcg./ml.
Escherichia coli O-111: 0.05 mcg./ml.
Escherichia coli T-7: 1.56 mcg./ml.
Krebsiella pneumoniae DT: 0.1 mcg./ml.
Proteus vulgaris IFO 3988: 1.56 mcg./ml.
Proteus morganii IFO 3848: 0.39 mcg./ml.
(2) Acute toxicity (mouse, intraperitoneal)
$LD_{50} \geqq 20$ g./kg.

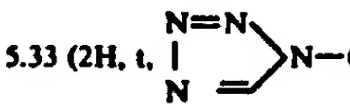
The acute toxicity data is for a 1:1 (molar) mixture of TTC and sodium carbonate.

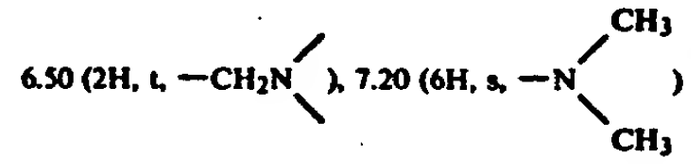
Reference Example 2

(1) To 400 g. of 2-(N,N-dimethylamino)ethylamine was added 2.4 l. diethyl ether and after cooling, a mixture of 400 g. of carbon disulfide and 4.0 l. of diethyl ether was added dropwise at 18° to 23° C. over a period of 1 hour. The mixture was stirred at that temperature for another hour, after which the resultant crystals of 2-(N,N-dimethylamino)-ethylaminecarbodithioic acid were recovered by filtration. Yield 695 g., yield 93.3%, m.p. 156° to 157° C.

To the crystals thus obtained above was added 4.4 l. of water and with stirring, 4.32 l. of 1 N-KOH was added dropwise at 8° to 13° C. over a period of 30 to 40 min., further followed by the dropwise addition of a mixture of 668 g. of methyl iodide and 6.68 l. of acetone at 0° to 5° C. over a period of 30 to 40 min. The mixture was stirred at a temperature of the same range for another 30 min. The acetone was distilled off under reduced pressure and the water layer was extracted with 3 l. of ethyl acetate and, then, 2 l. of the same solvent. The ethyl acetate layer was washed with 2 l. of a saturated aqueous solution of sodium chloride, dried over

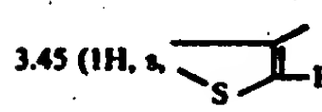
sodium sulfate and concentrated. The resultant crystals were recrystallized by the addition of 500 ml. of n-hexane. By the above procedure was obtained 575 g. of S-methyl-[2-(N,N-dimethylamino)]ethylamine carbodithioate, m.p. 61° to 62° C., in a yield of 75.5%. To 520 g. of the above crystals was added 1.05 l. of ethanol together with 190 g. of sodium azide and 2.1 l of pure water, and the mixture was heated under reflux for 3 hours, followed by the addition of a solution of 52 g. crystals of S-methyl-[2-(N,N-dimethylamino)]ethylamine carbodithioate in 100 ml. ethanol. The mixture was refluxed for 1 hour and, then, cooled to 20° C. To this was added 2.0 l. of pure water and, in nitrogen streams, the mixture was adjusted to pH 2 to 2.5 with concentrated hydrochloric acid. The ethanol was distilled off under reduced pressure and the residue was adsorbed on Amberlite IR-120 (H type) manufactured by Rohm and Haas Co., which was washed with pure water until acidity disappeared. The eluate obtained with 5% (weight/weight) aqueous ammonia was concentrated to obtain 350 g. crystals of 1-[2-(N,N-dimethylamino)ethyl]-5-mercapto-1H-tetrazole, m.p. 218° to 219° C., in a yield of 69.3%, N.M.R. (D_2O, with an equimolar amount of $NaHCO_3$ added, τ value):

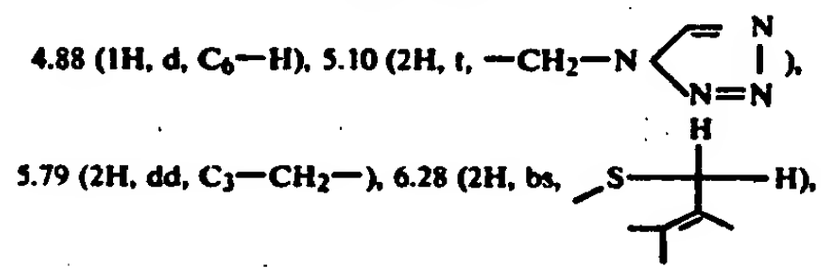




(2) To 2.6 l. of water was added 206 g. of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-acetyloxymethyl-3-cephem-4-carboxylic acid. Then, under stirring, 86.5 g. of 1-[2-(N,N-dimethylamino)ethyl]-5-mercapto-1H-tetrazole obtained in the above (1) and 42 g. of sodium hydrogen carbonate were added. The mixture was stirred at 65° C. for 75 min. and, then, cooled to 10° C. Following addition of 250 ml. of 5 N-HCl to adjust the mixture pH 2.0, the insolubles were recovered by filtration and rinsed with water. The filtrate and washings were combined, adjusted to pH 5.2 by the addition of sodium hydrogen carbonate and adsorbed on a column of 10 l. Amberlite XAD-II (100–200 mesh). The column was washed with 60 l. of water and, then, elution was carried out with 20% aqueous methanol and, then, 40% aqueous methanol. The fractions (11 l.) containing the desired compound were concentrated to 5 l. and passed columnwise over 300 g. of activated alumina (about 300 mesh) manufactured by Wako Pure Chemical Industries, Ltd. in Japan and over 100 ml. of Amberlite IR-120 (H type). The column was washed with water and the effluent and washings were pooled and concentrated to 2 l. The concentrate was cooled to 5° C. and stirred with 5 g. of activated carbon for 5 min. The activated carbon was filtered off and the filtrate was lyophilized to obtain 51.2 g. of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid.

N.M.R. (60 MHz D_2O, τ value):





(3) 0.5 l. of an aqueous solution containing 51.0 g. of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid obtained in above (2) was acidified to pH 2.0 with 12 N-HCl and cooled to 10° C. and stirred with 0.7 g. of activated carbon for 5 min. The activated carbon was filtered off and washed with 50 ml. of water. The filtrate and washings were combined and concentrated under reduced pressure to 228 ml. at an internal temperature of 15° to 17° C. The concentrate was filtered and insolubles filtered off were washed with water. The filtrate and washings were combined to obtain 238 ml. solution which contained 47.8 g. of the above carboxylic acid. To the solution was added 0.02 l. of acetone, followed by addition of 17.0 ml. of 12 N-HCl. Then, 0.7 l. of acetone was added over a period of 10 min. and, at 5° to 10° C., the mixture was stirred for 2 hours. Then, 0.7 l. of acetone was further added over a period of 30 min. The mixture was further stirred for 1 hour and allowed to stand overnight. The resultant crystals were recovered by filtration and washed with 100 ml. ×4 of acetone. The crystals were spread in a dish and allowed to dry in the air to remove most of the acetone. The crystals were then dried under reduced pressure (45 mmHg) for 1 hour. The crystals at this stage were composed of 77.7% of the above carboxylic acid, 10.8% of hydrogen chloride, 9.24% of water and 2.2% of acetone. The crystals were packed into a glass filter and pre-moistened nitrogen gas was passed through the bed of crystals for 4 hours to completely remove the acetone. The water content of the crystals at this stage was 16.4% (K.F. method). The crystals were further dried under reduced pressure (45 mmHg) to obtain 52.5 g. crystals of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride hydrate. The physical properties of this crystalline product were as follows. Water content (K.F. method) 3.12%; Purity on an anhydrate basis 99.5%; Crystalline, based on its powder X-ray diffraction pattern.

Examination of the product under a polarizing microscope revealed that it was crystalline.

Elemental analysis, $C_{18}H_{23}N_9S_3O_4.2HCl.H_2O$: Found C, 34.78; H, 4.51; N, 20.62; S, 15.31; Cl, 11.77. Calcd. C, 35.06; H, 4.41; N, 20.45; S, 15.60; Cl, 11.50.

Reference Example 3

(1) 5.0 l. of an aqueous solution containing 510 g. of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl}-1H-tetrazol-5-yl]thiometh-

yl-3-cephem-4-carboxylic acid obtained in Reference Example 2 (2) was acidified to pH 2.0 with 12 N-HCl and cooled to 1° C. and stirred with 7.0 g. of activated carbon for 5 min. The activated carbon was removed by filtration and rinsed with 500 ml. of water. The filtrate and washings were combined and concentrated under reduced pressure to 2.28 l. at an internal temperature of 15° to 17° C. The concentrate was filtered and washed again with water. The filtrate and washings, which totalled 2.38 l., contained 470 g. of the above carboxylic acid. To the filtrate was added 200 ml. of acetone, followed by addition of 170 ml. of 12 N-HCl. Then, 7 l. of acetone was further added over a period of 10 min. and the mixture was stirred at 5° to 10° C. for 2 hours. Thereafter, 7 l. of acetone was further added over a period of 30 min. The mixture was stirred for 1 hour and, then, allowed to stand overnight. The resultant crystals were collected by filtration and washed with 1 l.×4 of acetone. (A sample of the crystals was taken and dried in a desiccator at room temperature at 30 mmHg for 30 min. The water content as determined by K.F. method was 8.9%, with 2.2% of acetone attached. The water content calculated for $C_{18}H_{23}N_9O_4S_3.2HCl.3H_2O$ was 8.28%). The above crystals were transferred to a separate glass filter and nitrogen gas pre-moistened by passage through a water-containing scrubbing bottle (The water temperature was held at 25° to 30° C.) was passed through the bed of crystals at a rate of 8 l./min. for 6 hours. (A sample of the crystals thus obtained was separated and investigated for water content by K.F. method. The water content was 19.5%. The water content as calculated for $C_{18}H_{23}N_9O_4S_3.2HCl.8H_2O$ was 19.41%. This product contained no acetone at all and its powder X-ray diffraction pattern showed that it was crystalline). The above crystals were spread in a layer about 3 cm thick and dried at 30° C. at 5 mmHg for 1.5 hours. (The water content of a sample of these crystals was 17.2% as determined by K.F. method. The water content calculated for $C_{18}H_{23}N_9O_4S_3.2HCl.7H_2O$ was 17.41%). The above crystals were further dried under the same conditions for 1.5 hours, the water content as determined by K.F. method being 15.4%. (The water content calculated for $C_{18}H_{23}N_9O_4S_3.2HCl.6H_2O$ was 15.3% water). The crystals were further dried for 1.5 hours, the K.F. method water content being 13.3%. (The water content calculated for $C_{18}H_{23}N_9O_4S_3.2HCl.5H_2O$ was 13.08%). The above crystals were further dried for 1.5 hours, the K.F. method water content being 10.5%. (The calculated water content based on $C_{18}H_{23}N_9O_4S_3.2HCl.4H_2O$ was 10.75%). After drying for another 1.5 hours, 525 g. of crystals were obtained.

Water content (K.F. method) 8.50% (calcd. for $C_{18}H_{23}N_9O_4S_3.2HCl.3H_2O$=8.28%); powder X-ray diffraction pattern: crystalline; Cl content ($AgNO_3$ method) 10.6% (calcd. for $C_{18}H_{23}N_9O_4S_3.2HCl.3H_2O$=10.8%).

(2) The crystals obtained in (1) above were dried at 30° C., at 2 mmHg and in the presence of phosphoric anhydride, for 5 hours, whereby 510 g. of crystals were obtained.

Water content (K.F. method) 5.7% (calcd. for $C_{18}H_{23}N_9O_4S_3.2HCl.2H_2O$=5.68%); powder X-ray diffraction pattern: crystalline. I.R.(KBr)cm^{-1}: 1770(β-lactam), Sharp peaks characteristic of crystals appear at 1670, 1190(sh.) and 1170.

(3) The crystals obtained in (2) were dried at 30° C., at 2 mmHg and in the presence of phosphoric anhydride for 8 hours. By the above procedure was obtained 495 g. of crystals.

Water content (K.F. method) 3.12% (calcd. for $C_{18}H_{23}N_9O_4S_3.2HCl.H_2O$=2.92%); purity on anhydrate basis (high-speed liquid chromatography, on dichloride anhydrate basis) 99.5%; powder X-ray diffraction pattern: crystalline;

Elemental analysis, for $C_{18}H_{23}N_9O_4S_3.2HCl.H_2O$: Found C, 34.78; H, 4.51; N, 20.62; S, 15.31; Cl, 11.77. Calcd. C, 35.06; H, 4.41; N, 20.45; S, 15.60; Cl, 11.50.

$[\alpha]_D^{20}$(c=1%, H_2O)=+67.0°; residual solvent (acetone) 50 ppm. or less; Cl content ($AgNO_3$) 11.4%; calcd. 11.50%; λmax (H_2O) 258 mμ(ε19,500).

(4) 3 g. of the crystals obtained in (3) were dried at 5 mmHg and in the presence of phosphoric anhydride for 2 hours at 20° C. and 5 hours at 50° C., whereupon 2.6 g. of powdery product was obtained.

Water content (K.F. method) 0.3% (calcd. for $C_{18}H_{23}N_9O_4S_3.2HCl.0.1H_2O$=0.3%); powder X-ray pattern: amorphous; polarizing microscopy: crossed Nicol's prisms, interference colors on rotation of the slide, indicating optical anisotropy; Purity 99.6% (high-speed liquid chromatography, on a dihydrochloride anhydrate basis)

REFERENCE EXAMPLE 4

1.72 g. of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid obtained in Reference Example 2 (2) was suspended in 10 ml. of anhydrous methanol. To the suspension was added 6.20 ml. of N-hydrogen chloride anhydrous methanol solution, and the mixture was stirred to obtain a solution. The solution is portionwise added to 150 ml. of anhydrous ether to form precipitates.

The precipitates were collected by filtration, washed with anhydrous ether and dried under reduced pressure to obtain anhydrous 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride (i.e. TTC).

Elemental analysis as $C_{18}H_{23}N_9O_4S_3.2HCl$: Found C, 36.31; H, 4.26; N, 20.61. Calcd. C, 36.12; H, 4.21; N, 21.06.

EXAMPLE 1

250 g. of TTC hydrate as produced according to Reference Example 2 (3) was aseptically admixed with 44.3 g. of sterile particles-free sodium carbonate and the aseptic mixture was packed in portions of 250 mg. in terms of TTC into sterilized dry vials of 12 ml. capacity which were vacuum-sealed at 50 mmHg. The contents are dissolved quite readily upon addition of 3 ml. of distilled water.

EXAMPLE 2

By the same procedure as Example 1, 500 g. of TTC hydrate produced in Reference Example 3 (2) was mixed with 115.2 g. of potassium carbonate and the mixture was packed in portions of 500 mg. in terms of TTC into sterilized dry vials of 17 ml. capacity. The vials were vacuum-sealed at 50 mmHg.

EXAMPLE 3

250 g. of TTC hydrate as produced according to Reference Example 2 (3) was aseptically mixed with 70.2 g. of sterile particles-free sodium hydrogen carbonate and the mixture was packed into sterilized dry vials

of 17 ml. capacity in portions of 250 mg. based on the weight of TTC. The vials were vacuum-sealed in a vacuum of 2 mmHg.

EXAMPLE 4

250 g. of TTC hydrate produced in Reference Example 3 (3) was aseptically mixed with 35.2 g. of sterile particles-free magnesium carbonate and 125 mg. portions of the mixture in terms of TTC were respectively packed into sterilized dry vials of 9 ml. capacity. The vials were vacuum-sealed in a vacuum of 20 mmHg.

EXAMPLE 5

The procedure of Example 4 was repeated except that 83.6 g. of calcium carbonate was used in lieu of 35.2 g. of magnesium carbonate. By this procedure was obtained an antibiotic composition.

EXAMPLE 6

The procedure of Example 3 was repeated except that 250 g. of TTC hydrate prepared in Reference Example 4 were used in lieu of 250 g. of TTC hydrate produced in Reference Example 2 (3). By this procedure was obtained vacuum-sealed vials containing an antibiotic composition.

EXAMPLE 7

The procedure of Example 1 was repeated except that 250 g. of any one of the TTC produced in Reference Example 4 and the TTC hydrates produced in Reference Example 3 (2) and 3 (4) was used in lieu of 250 g. TTC hydrate produced in Reference Example 2 (3).

Experiment 1

The solution produced according to Example 1 was subcutaneously administered to mice infected with the following pathogenic microorganisms to ascertain the ED_{50} values (mg. of TTC/kg. of mouse).

ED_{50} values

Staphylococcus aureus:
308 A-1
7.14 (mg./kg.)
Escherichia coli:
0-111
0.074 (mg./kg.)
Proteus vulgaris: IFO-3988
1.32 (mg./kg.)

Experiment 2

250 mg. of TTC hydrate obtained in Reference Example 2 (3) was admixed with 50 mg. of sodium carbonate and the mixture was packed into a vial of 12 ml. capacity which was then vacuum-sealed in a vacuum of 50 mmHg. The product was designated Sample A. On the other hand, a mixture of 250 mg. of TTC hydrate obtained in Reference Example 2 (3) and 50 mg. of sodium carbonate was packed into a vial of 12 ml. capacity. This vial was not vacuum-sealed and designated Sample B. 250 mg of TTC hydrate obtained in Reference Example 2 (3) alone was filled into a 12 ml. vial, which was not vacuum-sealed and designated Sample C. To each of the Samples was added 3 ml. of distilled water and the times of dissolution were measured. The colors of the Samples 3 hours after dissolution were also evaluated.

Sample	Dissolution time	Color 3 hours after dissolution
A	15 sec.	Yellow to yellowish tan
B	70 sec.	Yellow to yellowish tan
C	180 sec.	Reddish yellow

Provided that, in dissolution, Samples A and B were allowed to stand, while Sample C was shaken vigorously.

EXPERIMENT 3

1 ml. portions of each of the following injectable fluids were injected into the vastus lateralis muscles of rabbits and, after 24 hours, the animals were killed. The muscles were taken and dissected to examine the degrees of injury (local reactions) by the naked eye. The findings were scored according to the following scheme.

Score	Symptom
0	No discernible gross reaction
1	Slight hyperemia
2	hyperemia and moderate discoloration
3	Discoloration
4	Brown degeneration or necrosis with hyperemia
5	Widespread necrosis

The results are set forth below:

Composition	Local reaction Single administration, after a day
TTC hydrate 250 mg.*	4
TTC hydrate 250 mg.* + anhydrous sodium carbonate 50 mg.	0
TTC hydrate 250 mg.* + sodium hydrogen carbonate 86 mg.	0

*TTC hydrate used in the above compositions were obtained in Reference Example 2 (3).

The powders of each composition were respectively dissolved in 2 ml. of distilled water and the local reactions were investigated.

What is claimed is:

1. A vacuum-sealed vial, which contains a solid antibiotic composition comprising 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride or its hydrate and sodium carbonate or sodium hydrogen carbonate, the ratio of the hydrogen chloride moiety of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride or its hydrate relative to said sodium carbonate or sodium hydrogen carbonate being substantially 1:1 to 2 equivalents, the pressure in the vial being in the range of from 0 to 300 mmHg.

2. A vacuum-sealed vial as claimed in claim 1, wherein sodium carbonate is contained in the composition.

3. A vacuum-sealed vial as claimed in claim 1, wherein sodium hydrogen carbonate is contained in the composition.

4. A vacuum-sealed vial as claimed in claim 1, wherein 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)-ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride hydrate, of which the water content is substantially 1 to 4 mols per mol of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-}1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride moiety, is contained in the composition.

5. A vacuum-sealed vial as claimed in claim 4, wherein the water content is substantially 1 to 2 mols per mol of β7-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride moiety.

6. A vacuum-sealed vial, which contains a solid antibiotic composition comprising 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride hydrate, of which the water content is substantially 1 to 4 mols per mol of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}-thiomethyl-3-cephem-4-carboxylic acid dihydrochloride moiety, and sodium carbonate, the amount of sodium carbonate being substantially 1 to 2 mols per mol of said hydrate, the pressure in the vial being in the range of from 0 to 300 mmHg.

7. A vacuum-sealed vial as claimed in claim 6, wherein the water content is substantially 1 to 2 mols per mol of 7β-[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-{1-[2-(N,N-dimethylamino)ethyl]-1H-tetrazol-5-yl}thiomethyl-3-cephem-4-carboxylic acid dihydrochloride moiety.

* * * * *